REMARKS

The Examiner is thanked for the due consideration given the application. The specification has been amended to insert headings.

Withdrawal of the restriction requirement is noted with appreciation.

Claims amendments

Claim 20 has been amended. The new claim 20 now recites an enzymatically active GFAT comprising an *E. coli* or human sequence wherein a tag sequence is inserted in specific regions.

Claims 21-25 have been cancelled.

Claim 27 has been amended to remove the term "approximately".

Claim 29 has been amended to replace the term "corresponding to" by the terms "consisting of".

Claim 30 has been cancelled.

Claim 31 has been amended to remove the recitation "***or by its complementary sequence, or being derived from said sequence by mutation, insertion or deletion of at least one nucleotide, provided that said nucleotide sequence codes for a enzymatically-active protein***"

Claim 34 has been amended to replace the term "corresponding to" by the term "consisting of".

Claim 35 has been amended to remove the terms "approximately" and "in an enzymatically-active form".

Claim 36 has been amended to restrict the subject matter to a composition comprising an enzymatically active GFAT protein as claimed in claim 1, in association with fructose 6-phosphate, Tris(2-carboxyethyl) phosphine and glycerol.

Also, terms "approximately" have been canceled.

Further, terms "-00°C to -20°C" have been replaced by terms "-100°C to -20°C" in accordance with the recitation of the specification page 10, line13.

Claims 37-38 have been canceled.

No new matter is believed to be added to the application by these amendments.

Rejection Under 35 USC §101

Claims 20-32 and 36 have been rejected under 35 USC §101 as being directed to non-statutory subject matter. This rejection is respectfully traversed.

The Official Action asserts that the claims as written do not sufficiently distinguish over nucleic acids, proteins and enzymes as they exist naturally because the claims do not particularly point out any non-naturally occurring differences between the claimed products and the naturally occurring products.

However, claims 20, 26-29, 31, 32 and 36 have been amended to add the term "isolated", which clearly distinguishes the claimed invention from naturally occurring products. The present

invention thus clearly falls under the statutory *aegis* of 35 USC §101.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Rejection Under 35 USC §112, Second Paragraph

Claims 20-24, 27-30 and 32-38 have been rejected under 35 USC §112, second paragraph as being indefinite. This rejection is respectfully traversed.

The Official Action asserts that claim 20 recites the phrase "enzymatically-active protein", which renders the claims indefinite. However, the claims have been amended to recite that the claimed enzymatically active protein possesses a glutamine:fructose-6-phosphate amidotransferase (GFAT) activity.

Also, claims 29 and 34 have been clarified by replacing the terms "corresponding to" by the terms "consisting of".

Claims 21, 22, 24 and 37-38 have been cancelled, thereby rendering the rejection of these claims moot.

The claims are thus clear, definite and have full antecedent basis.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Rejection Under 35 USC §112, First Paragraph

Claims 20-24 and 27-38 have been rejected under 35 USC §112, first paragraph as failing to comply with the written description

requirement and as not being enabled. This rejection is (or rejections are) respectfully traversed.

It is respectfully submitted that the instant claims are in compliance with 35 USC §112, first paragraph.

Newly presented claim 20 now recites an isolated protein possessing a GFAT activity, wherein a tag sequence is inserted between two contiguous amino acids, said amino acids being contained in specific regions, such as:

- the region delimited by amino acids in position 220 and 230 of the SEQ ID NO 13,
- the region delimited by amino acids in position 298 and 306 of the SEQ ID NO: 2,
- the region delimited by amino acids in position 299 and 307 of the SEQ ID NO: 4,
- the region delimited by amino acids in position 316 and 324 of the SEQ ID NO: 6.

The specification illustrates the insertion of a specific tag sequence in SEQ ID NO 2, 4, and 6.

Concerning the GFAT protein of *E. coli* (SEQ ID NO 13), the Inventors have published after the filing date of the present application data demonstrating that insertion of a tag sequence (6xHIS tag) between the amino acids 224 and 225, does not affect the GFAT activity of such a protein. Indeed, as mentioned in table 1, page 49 of Richez et al. (Richez et al., *Protein Expression and Purification* 54 (2007) 45-53; enclosed with this

reply), 6xHis tagged GlmS protein (having GFAT activity) has an activity similar to those of the natural (untagged) GlmS protein.

This article corresponds to the present invention, in which some complementary data have been added.

Thus, at the filing date, even if some results have been confirmed after, the Inventors have clearly and unambiguously described the regions in GFAT (prokaryote and human) proteins where a tag can be inserted.

From the recitation of newly amended claim 20, any skilled person would be able to reproduce the invention, since such a person with ordinary skill would understand which protein is used, and which region should be targeted for inserting a tag sequence.

Also, regarding the DNA and proteins, although these molecules have a completely different structure, in Biology these molecules are known to form a global genus that "contains and diffuses the biological information".

Any skilled person knows that DNA molecule, even if it does not have an enzymatic activity, encode for another molecule, i.e., the protein, which have said enzymatic activity.

Thus, a skilled artisan would understand that DNA molecule SEQ ID NOs 7, 9 and 11 code for protein with enzymatic activity SEQ ID NOs 8, 10 and 12. Moreover, from the teaching of the experimental procedure, the skilled person would carry out the invention, regarding the nucleic acid molecules or proteins.

Therefore, from the instant claims, a person with ordinary skill would conclude that at the filing date the applicant was in possession of the invention.

In paragraph 9 the Official Action argues that the specification does not provide sufficient guidance and predictability information to reproduce the invention as claimed.

The Official Action acknowledges that the specification provides sufficient guidance and predictability to reproduce the invention regarding SEQ ID NOs 8, 10 and 12, and regarding the nucleic acid sequences coding them, i.e. SEQ ID NOs 7, 9 and 11.

The claims have been consequently modified with respect to the comments in the Official Action, and the newly amended claims 20, 26-29, 31-36 satisfy to the criteria of 35 USC § 112, first paragraph.

Newly amended claim 20 now recites an isolated protein possessing a GFAT activity, wherein a tag sequence is inserted between two contiguous amino acids, said amino acids being contained in specific regions, such as:

- the region delimited by amino acids in position 220 and 230 of the SEQ ID NO 13,
- the region delimited by amino acids in position 298 and 306 of the SEQ ID NO: 2,
- the region delimited by amino acids in position 299 and 307 of the SEQ ID NO: 4,

- the region delimited by amino acids in position 316 and 324 of the SEQ ID NO: 6.

The specification illustrates the insertion of a specific tag sequence in SEQ ID NO 2, 4, and 6, and Richez et al. provide illustration for the insertion of a tag sequence in *E. coli* GFAT sequence.

From the teaching of the specification, the skilled person has some guidance and predictability information to reproduce the invention as claimed, in newly presented claims.

Indeed, the present invention teaches that a tag sequence can be inserted between two amino acids contained in a particular region of a GFAT protein. It is particularly illustrated by the specific insertion of 6xHis tag in GFAT1, GFAT2 and GFATalt human sequences.

The invention as instantly claimed includes the possibility to insert a tag sequence in a sequence defined by an amino acid sequence of 10 amino acids of SEQ ID NO 13, 8 amino acids of SEQ ID NO 2, 8 amino acids of SEQ ID NO 4, and 8 amino acids of SEQ ID NO 6.

These regions are well characterized since the specification gives the position of the beginning and the end of the interval, in a specific sequence. These regions have been characterized from the analysis of the crystal structure of *E. coli* GlmS (GFAT) protein, published by Teplyakov et al. (Teplyakov et al., *J. Mol.*

Biol 313 (2001) : 1093-1102; enclosed with this reply); structure which was known at the filing date.

In Teplyakov et al., the authors have elucidated the tri-dimensional structure of the bacterial GFAT protein, GlmS. In particular, the authors have demonstrated that the isomerase and glutaminase domain form separated globular structure linked by the amino acids of the region comprised between amino acid in position 240 to 280. In Figure 7, it is also demonstrated that the isomerase and glutaminase activity need to be close to ensure the full activity of the protein.

Knowing the teaching of Teplyakov et al. regarding the structure of GlmS, the specification gives sufficient guidance to indicate to a skilled person that insertion of a tag sequence in the linking region (240-280) would separate gluataminase and isomerase activity, and therefore would reduce or inhibit GFAT activity of the enzyme.

Thus, the skilled person would understand that only the region disclosed by the claimed invention could accept an insertion of a tag sequence without perturbing the GFAT activity.

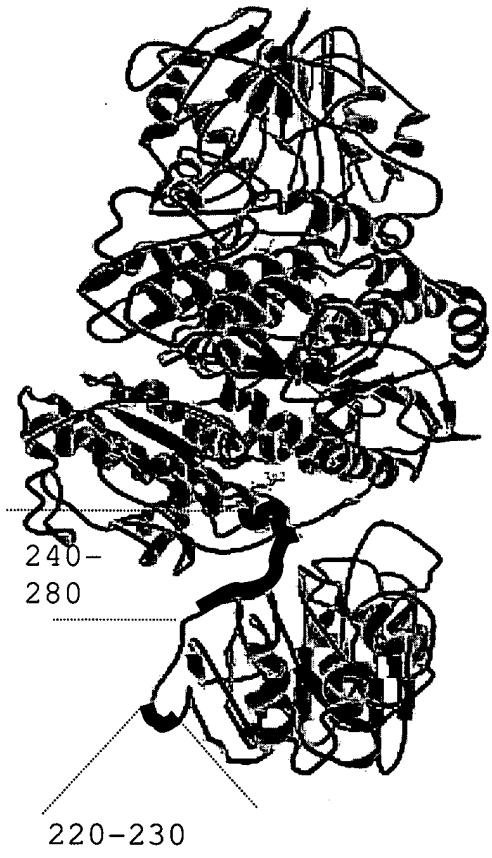
The invention as claimed gives sufficient guidance and predictability to the skilled person to insert a tag sequence in a region that, if modified, would not inhibit or abolish the GFAT activity.

Indeed, as presented in the following Figure 1, which represent the 3D structure of GlmS, the region corresponding to

amino acids 220-230 of GlmS is spatially separated from the linking sequence 235-280.

Figure 1:

3D structure of dimeric GlmS. Regions 240-280 and 220-230 are represented with black bold line (adapted from Teplyakov et al.)



So, with the knowledge of the Teplyakov structure, the specification gives sufficient guidance to a person with ordinary skilled to understand why the 220-230 region is selected instead of the 240-280 region.

Moreover, before the filing date, it was known from the related art that if a first insertion between two specific amino acids of a tag sequence can be made in a region having a particular structure, others insertions of a tag sequence can be

made between two other amino acids in the same region having a particular sequence, without interfering with the properties conferred by the first insertion.

This concept can be illustrated by Biondi et al. (Biondi et al., *Nucleic Acid Research*, 26(21) (1998) 4946-4952; enclosed with this reply). In this document, the authors have randomly inserted GFP tag sequence in the regulatory subunit of cAMP dependent kinase, and have selected different recombinants proteins. In the document, the authors demonstrate that insertions of GFP sequence between two amino acids contains in the α-helix present after the cAMP binding site A, all the tagged protein have the same properties (clones 89, 26, 34 and 35; see Figure 5 and page 4952).

It is important to note that, in this article, the insertion modifies the initial function of the protein, but demonstrates that insertion in the same α-helix does not change the modification conferred by the GFP insertion.

Moreover, without information provided by the invention as claimed, a skilled person would obviously incited to combine the recitation of Chang et al. and Ferguson et al. and would obviously incited to provide a **non-functional** 6xHis tag GFAT protein (region 240-280), which does not correspond to the GFAT protein of the present invention as claimed, since this GFAT protein has lost its catalytic activity.

With the recitation of the invention as claimed the skilled person had some guidance (where to insert tag sequence) and predictability information (example demonstrating the functionality of the His tagged GFAT) to reproduce the invention.

Thus, from the teachings of the disclosure of the present invention, a skilled person would be able to reproduce the invention as claimed, i.e. to produce GFAT protein having a tag sequence inserted between two contiguous amino acids, said amino acids being contained in specific regions, such as:

- the region delimited by amino acids in position 220 and 230 of the SEQ ID NO 13,
- the region delimited by amino acids in position 298 and 306 of the SEQ ID NO: 2,
- the region delimited by amino acids in position 299 and 307 of the SEQ ID NO: 4,
- the region delimited by amino acids in position 316 and 324 of the SEQ ID NO: 6.

Without undue burden, the skilled artisan would applied the protocol described in the specification for the specific insertion and would easily reproduce such a strategy to produce the other claimed protein.

As a consequence, from the teaching of the invention as claimed, the person with an ordinary skill has sufficient guidance and predictability information to provide the claimed GFAT.

The present invention is thus sufficiently described in the disclosure, and one of ordinary skill can practice the present invention without recourse to undue experimentation.

This rejection is (or rejections are) believed to be overcome, and withdrawal thereof is respectfully requested.

CONCLUSION

The Examiner is thanked for considering the Information Disclosure Statement filed January 6, 2006 and for making the initial PTO-1449 form of record in the application.

The rejections are believed to be overcome, obviated or rendered as moot and no issues remain. The Examiner is respectfully requested to place the application in condition for allowance and to issue a Notice of Allowability.

The Commissioner is hereby authorized in this, concurrent, and future submissions, to charge any deficiency or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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APPENDIX:

The Appendix includes the following articles:

☒ - Richez et al., *Protein Expression and Purification* 54 (2007) 45-53.

☒ - Biondi et al., *Nucleic Acid Research*, 26(21) (1998) 4946-4952

☒ - Teplyakov et al., *J. Mol. Biol* 313 (2001) : 1093-1102